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A new species of *Ninia* (Serpentes, Colubridae) from western Ecuador and revalidation of *N. schmidti*

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Abstract

We describe a new species of *Ninia* Baird & Girard, 1853 endemic to the cloud forests of northwestern Ecuador. The new species has previously been confused with *N. atrata* (Hallowell, 1845) and *N. teresitae* (Angarita-Sierra and Lynch 2017) but is genetically most closely related to a third species of *Ninia* endemic to the Chocoan–Tumbesian transition area of western Ecuador. We revalidate the name *N. schmidti* (Jan, 1862), designate a neotype, and provide a diagnosis of the taxon and a description of its hemipenial morphology based on new material. The new and revalidated species can easily be identified from one another and from other trans-Andean South American *Ninia* based on ventral and subcaudal scale counts, hemipenial morphology, and coloration of the supralabials, throat, and belly. Finally, we remove *N. atrata* from the herpetofaunal list of Ecuador.

Key Words

Dipsadidae, coffee snakes, snake, Squamata, taxonomy

Introduction

The Variable Coffee Snake *Ninia atrata*, as currently understood, is a small blackish snake widely distributed throughout the lowlands and adjacent foothills of the Andes in northern South America, including much of western Ecuador (Angarita-Sierra 2009; Ingrasci 2011). Aptly named, the Variable Coffee Snake exhibits remarkable intraspecific variation in coloration, squamation, and morphometrics, notably in the shape and color of the nuchal collar (Angarita-Sierra 2009). Much of what is accepted about the current limits and variation within this species was presented in the seminal work on the snakes of the genus *Ninia* by Dunn (1935), who considered that only one species of *Ninia* inhabited South America. Later, Parker (1940), Burger and Werler (1954), and more recently, Angarita-Sierra (2009, 2014, 2018), Ingrasci (2011), and Angarita-Sierra and Lynch (2017), have challenged this view and provided evidence that northern South America is inhabited by more species of coffee snakes, all previously subsumed under *N. atrata*, now accepted to be a species complex.

The *Ninia atrata* complex also occurs in Ecuador, where at least two names other than *N. atrata* were applied to specimens now subsumed under this complex: *Streptophorus sebae schmidti* Jan, 1862 and *N. spilogaster* Peters, 1881. The holotype of *S. sebae schmidti* (deposited at ZMH) is labeled as having originated from Guayaquil, Guayas province, but is now destroyed (Jakob Hallermann, pers. comm. to AA). The type locality of *Ninia spilogaster* is "Ecuador" and the holotype (presumably at Museum für Naturkunde, Berlin; ZMB) could not be located (Mark-Oliver Rödel, pers. comm. to AA). Two additional names were erected for snakes in this group, *Streptophorus drozii* Duméril, Bibron & Duméril, 1854 and *Streptophorus lansbergi* Duméril, Bibron & Duméril, 1854, with holotypes MNHN-RA 3444 from "La Nouvelle-Orléans" and MNHN-RA 3446 from Caracas,

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respectively. Dunn (1935) considered these names to be synonyms of *N. atrata*. Thus, until 2018, only two species were recognized in Ecuador: *N. atrata* west of the Andes and *N. hudsoni* (Parker, 1940) east of the Andes. Using characters of coloration, lepidosis, and hemipenial morphology, Angarita-Sierra (2018) demonstrated that many specimens previously labeled as *N. atrata* from the Chocó biogeographic region in western Ecuador corresponded to *N. teresitae*, a trans-Andean species from Colombia (Angarita-Sierra and Lynch 2017). However, the *N. atrata* puzzle in Ecuador was not fully solved. The limits of Ecuadorian populations of *N. atrata* were not examined using molecular characters and populations occurring in the cloud forests or along the Chocoan-Tumbesian transition area were not examined.

Here, we attempt to fill-in this information gap by providing a systematic review of the unsampled populations using both molecular and morphological characters. As a result, we describe a new species of *Ninia* endemic to the cloud forests of northwestern Ecuador, revalidate the name *N. schmidti*, and remove *N. atrata* from the herpetofaunal list of Ecuador.

Materials and methods

Ethics statement

This study was carried out in strict accordance with the guidelines for use of live amphibians and reptiles in field research (Beaupre et al. 2004) compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL) and the Society for the Study of Amphibians and Reptiles (SSAR). All procedures with animals (see below) were reviewed by the Ministerio del Ambiente, Agua y Transición Ecológica (MAATE) and specifically approved as part of obtaining the following field permits for research and collection: MAE-DNB-CM-2018-0105 and MAATE-DBI-CM-2022-0245 (granted to Universidad San Francisco de Quito). Specimens were euthanized with 20% benzocaine, fixed in 10% formalin or 90% ethanol, and stored in 70% ethanol. Voucher specimens were deposited at Museo de Zoología de la Universidad San Francisco de Quito (ZSFQ). Specimens labeled JMG, SC, SCA, TH, and THI were also deposited at ZSFQ.

Morphological data and collection acronyms

Terminology for *Ninia* cephalic shields follows Angarita-Sierra (2009), diagnoses and descriptions generally follow Angarita-Sierra and Lynch (2017), and ventral and subcaudal counts follow Dowling (1951). We examined fluid-preserved specimens from the herpetology collections at Zoologisches Museum Hamburg (**ZMH**), Fundación Herpetológica Gustavo Orcés (**FHGO**), and Museo de Zoología de la Universidad San Francisco de Quito (**ZSFQ**) (Suppl. material 1). Abbreviations are as follows: caudal length (**CL**); head length (**HL**); head width (**HW**); snout-vent length (**SVL**); total length (**TL**).

Preparation of hemipenial morphology

The hemipenes were removed and prepared from museum specimens using the procedures of Pesantes (1994) and Zaher (1999). Hemipenial terminology is based on Dowling and Savage (1960), Zaher (1999), and Myers and MacDowell (2014).

Sampling and laboratory techniques

Genomic DNA was extracted from 96% ethanol-preserved tissue samples (liver, muscle, or scales) using either a guanidinium isothiocyanate extraction protocol (Peñafiel et al. 2020) or a modified salt precipitation method based on the Puregene DNA purification kit (Gentra Systems). The nucleotide sequences of the primers and the PCR conditions applied to each primer pair are detailed in Appendix 1. PCR products were cleaned with either ExoSAP-IT (Affymetrix, Cleveland, OH) or Exonuclease I and Alkaline Phosphatase (Illustra ExoProStar by GE Healthcare) before they were sent to Macrogen Inc (Seoul, South Korea) for Sanger sequencing. All PCR products were sequenced in both forward and reverse directions with the same primers that were used for amplification. We generated sequence data for samples marked with an asterisk in Suppl. material 2. The sequences were deposited in GenBank and the accession numbers are listed in Suppl. material 2.

DNA phylogenetic analyses

A total of 49 DNA sequences were used to build a phylogenetic tree of the genus *Ninia*, of which 37 were generated during this work and 12 were downloaded from GenBank. Of these, 15 sequences are 418–776 bp long fragments of the 16S gene, 13 are 313–775 bp long fragments of the CYTB gene, 12 are 585–647 bp long fragments of the ND4 gene, and 10 are 795 bp long fragments of the RAG1 gene. New sequences were edited and assembled using the program Geneious Pro™ 2021.1.1 (Drummond et al. 2021) and aligned with those downloaded from Genbank (Suppl. material 2) using MAFFT v.7 (Katoh and Standley 2013) under the default parameters. Gene fragments were concatenated into a single matrix with 10 partitions, one per non-coding gene and three per protein coding gene corresponding to each codon position. The best partition strategies along with the best-fit models of evolution were obtained in PartitionFinder 2.1.1 (Lanfear et al. 2016) under the Bayesian Information Criterion.

Phylogenetic relationships were assessed under a Bayesian inference (BI) approach in MrBayes 3.2.0

(Ronquist and Huelsenbeck 2013). Four independent analyses were performed to reduce the chance of converging on a local optimum. Each analysis consisted of 6,666,667 generations and four Markov chains with default heating settings. Trees were sampled every 1,000 generations and 25% of them were arbitrarily discarded as "burn-in." The resulting 5,000 trees saved per analysis were used to calculate the posterior probabilities (PP) for each bipartition in a 50% majority-rule consensus tree. We used Tracer 1.6 (Rambaut et al. 2022) to assess convergence and effective sample sizes (ESS) for all parameters. Additionally, we verified that the average standard deviation of split frequencies between chains and the potential scale reduction factor (PSRF) of all the estimated parameters approached values of ≤ 0.01 and 1, respectively.

Distribution maps and ecological niche models

We present ranges of occurrence for the three species of *Ninia* known to occur in western Ecuador. Presence localities are derived from museum vouchers (Suppl. material 1), photographic records (iNaturalist), and the literature (all summarized in Suppl. material 3). For each species, both a binary environmental niche model (ENM) and a dot map is presented. These ENMs estimate potential areas of distribution based on observed presences and a set of environmental predictors (Elith and Leathwick 2009). To delimit the occupancy areas and the potential species distribution, we used the BAM diagram proposal (Soberón and Peterson 2005; Peterson et al. 2011). To create the models, we used presence localities listed in Suppl. material 3, 19 bioclimatic variables from Worldclim 1.4 (Hijmans et al. 2005), and Maxent 3.4.1k, an algorithm based on the principle of maximum entropy (Phillips et al. 2006; Elith et al. 2011; Renner and Warton 2013).

For the first explorative exercise, we used the 19 climate layers from the WorldClim project and assessed which variables were the most important for the model, according to the Jackknife test calculated in MaxEnt (Royle et al. 2012). Correlated environmental variables ($r < 0.8$) were identified using the PEARSON correlation test of PAST 3. In a second modelling exercise, we used the locality records for each species and the variables identified in the first approach to generate the species distribution. 5,000 iterations were specified to the program with clamping and no extrapolation. All other parameters in MaxEnt were maintained at default settings. To create the binary environmental niche models, suitable areas were distinguished from unsuitable areas by setting a **minimum training presence** threshold value. The logistic format was used to obtain the values for habitat suitability (continuous probability from 0 to 1), which were subsequently converted to binary presence-absence values based on the established threshold value, defined herein as **the minimum training presence**. The convergence threshold was set to 10^{-5}, maximum iterations to 500, and the regularization parameter to "auto."

Rationale for definition of species-level candidate taxa

We here recognize species limits following an integration by congruence approach (Padial et al 2010) based on the intersection of evidence from three or more independent taxonomic characters. We follow the general species concept of de Queiroz (2007) in defining species as independent evolutionary if two or more independent lines of evidence support their distinctness. We start the species delimitation procedure by seeking mitochondrial clades divergent from other mitochondrial clades by sequence divergences > 3% in a 700 bp fragment of the CYTB gene, given that this degree of divergence has been found to correspond to species-level units in other *Ninia*, such as the species pairs *N. atrata–N. hudsoni* (Ingrasci 2011). We then tested if the individuals belonging to these mitochondrial lineages can be unambiguously diagnosed based on: (1) lepidosis; (2) hemipenial morphology; (3) coloration; and (4) size. Finally, we evaluated if these mitochondrial lineages are congruent geographically. Thus, in our species delimitation approach, we consider groups of individuals as species if they (1) form a monophyletic group based on mtDNA; (2) differ from other such groups by > 3% sequence divergence (mean p-distance); (3) are diagnosable based on external characters of coloration and lepidosis; (4) are diagnosable based on hemipenial morphology; and (5) are congruent ecologically and biogeographically.

Results

Molecular phylogeny

Selected partitions and models of evolution are presented in Table 2. We consider strong support for a clade when Bayesian analyses yield posterior probability values > 95%, following Felsenstein (2004). The topology and support (Fig. 1) of our phylogenetic tree renders *Ninia atrata* as paraphyletic with respect to all other included *Ninia* species.

The sample of *Ninia atrata* from the lowlands of the Río Magdalena valley in Caldas department in Colombia (MHUA 14452) is strongly supported as sister to all other *Ninia* species included. *Ninia maculata* (Peters, 1861) is the moderately supported sister species of a clade that includes the remainder congeners. The two included samples of *N. teresitae* (one from Colombia, MHUA 15130, and one from Ecuador, KU 218424) form a monophyletic group (red clade in Figs 1 and 2) sister to the members of the *N. atrata* complex. The Ecuadorian sample of *N. hudsoni* is recovered as sister to the two cis-Andean samples of *N. atrata*. A species of *Ninia* endemic to southwestern Ecuador (purple clade) is the strongly supported sister taxon of another new species endemic to the cloud forests of northwestern Ecuador (yellow clade).

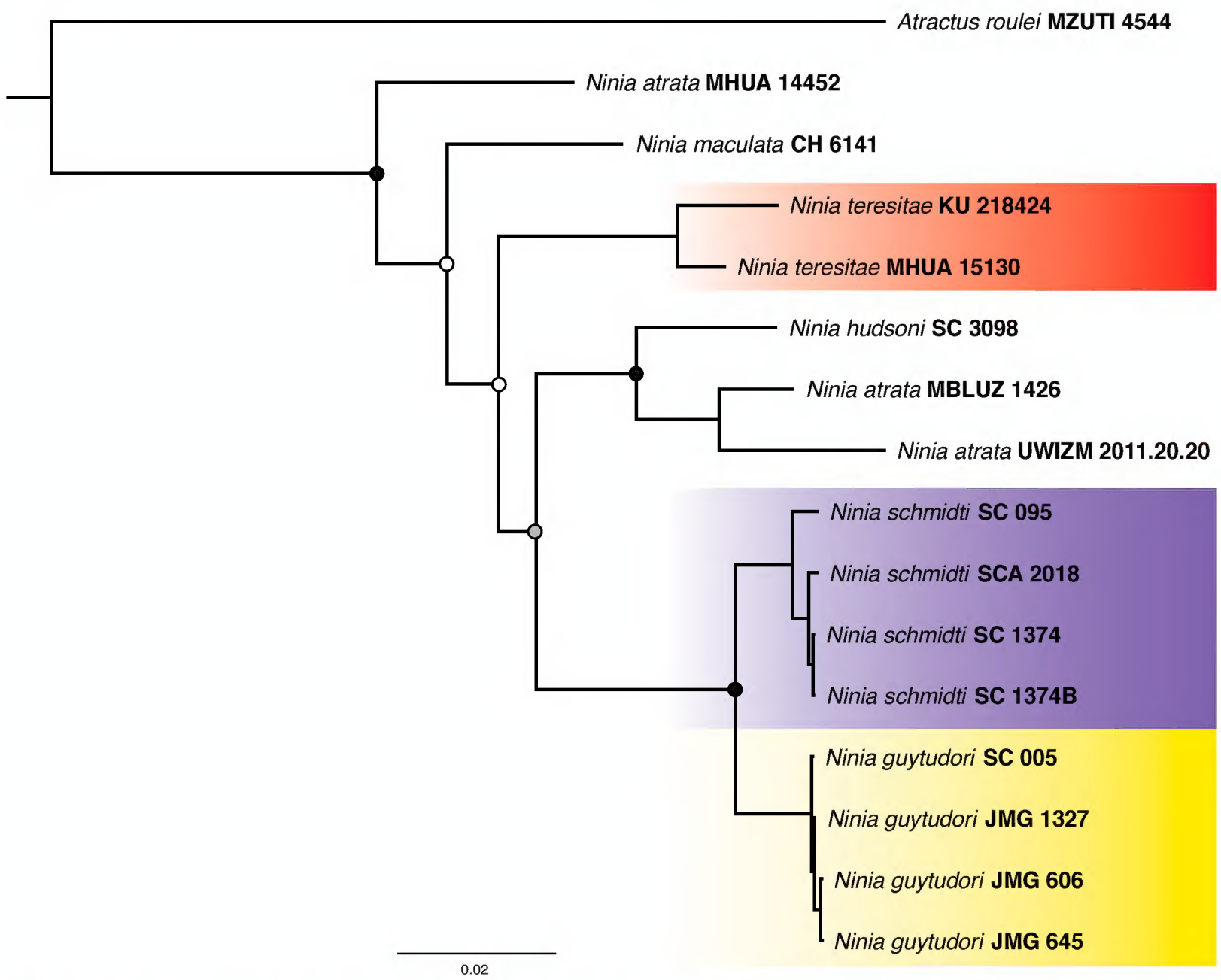

Figure 1. Phylogenetic relationships within *Ninia* inferred using a Bayesian inference and derived from analysis of DNA gene fragments 16S, CYTB, ND4, and RAG1. Support values on intra-specific branches are not shown for clarity. Voucher numbers for sequences are indicated for each terminal. Black dots indicate clades with posterior probability values from 95–100%. Grey dots indicate values from 70–94%. White dots indicate values from 50–69% (values <50% not shown). Colored clades correspond to the species' distribution presented in Fig. 2.

We restrict the name *Ninia schmidti* comb. nov. to the purple clade in Fig. 1, which includes samples from throughout the species area of distribution, based on the type locality of *Streptophorus sebae schmidti*: Guayaquil, where no other member of the genus is known to occur. ZMH R10390, housed also at the Zoologisches Museum Hamburg, is from the type locality and morphologically resembles the other seven specimens referred to this taxon (Suppl. material 1) in having ventral surfaces obscured by dark brown pigment particularly along the posterior edge of each ventral scale and throat and chin shields obscured by dark brown pigment. This description is not shared by members of the yellow clade but is present in members of *N. teresitae*. However, the low number of subcaudals (=46) and the locality of ZMH R10390 rules out allocation to that taxon. To further clarify this association and to bring stability to the taxonomy of *Ninia* in Ecuador, we designate ZMH R10390 as the neotype of *N. schmidti* comb. nov.

Systematic accounts

Species delimitation and the distinction between species-level and intraspecific variation is a complex topic (Carstens et al. 2013; Burbrink et al. 2022). In this instance, we name and provide descriptions only for species that are monophyletic in our molecular phylogeny and share diagnostic features of their coloration pattern, lepidosis, and biogeography. Future studies might elucidate species boundaries based on explicit species-delimitation analyses. Based on these delimitation criteria, which follow the general lineage species concept of de Queiroz (2007), we describe a new species of *Ninia* and revalidate *N. schmidti* comb. nov.

Ninia guytudori sp. nov.

https://zoobank.org/6A72861B-F4CF-465E-B73C-E11790A65DE8

Figs 3, 4a, 5a–d, 6, 7b

Holotype. JMG 1327 (Figs 3, 4a, 5a–b), adult female collected by Alejandro Arteaga on July 27, 2017 at road to Mindo, Pichincha province, Ecuador (-0.02825, -78.76189; 1676 m).

Paratopotype. SC 005 (Fig. 5c, d), juvenile male with the same data as the holotype.

Proposed standard English name. Tudors's Coffee-Snake.

Proposed standard Spanish name. Culebra cafetera de Tudor.

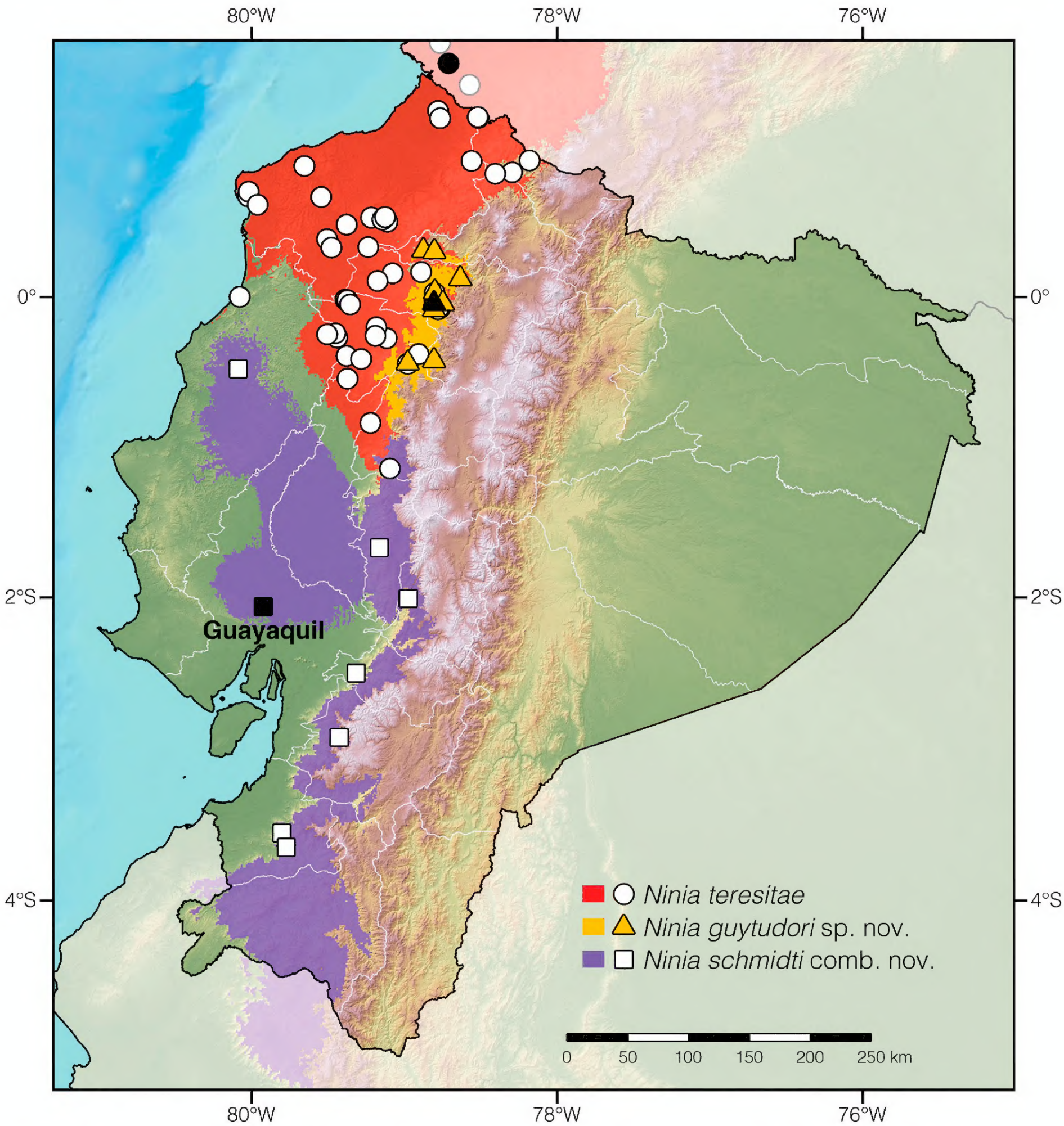

Figure 2. Distribution of species of *Ninia* in western Ecuador. Black symbols represent type localities. Each colored area is a geographic representation of the suitable environmental conditions for one of the clades recovered in the phylogeny of Fig. 1.

Diagnosis. *Ninia guytudori* sp. nov. is placed in the genus *Ninia*, as diagnosed by Dunn (1935), based on phylogenetic evidence (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 19/19/19 keeled dorsals; (2) two postoculars or none in SC 005; (3) loreal 1.6–1.7 × longer than high; (4) temporals 1+2; (5) seven or eight supralabials, usually fourth and fifth contacting orbit; (6) seven or eight infralabials, first four or five contacting chin shields; (7) two rows of chin shields; (8) two or three preventrals; (9) 130–138 ventrals in males, 144 in the single female; (10) 48–51 subcaudals in males, 44 in the single female; (11) dorsal ground color uniformly black with a white nuchal collar that connects to a white lip band forming a bridle (Fig. 6); (12) ventral surfaces uniformly immaculate white (Fig. 3b); (13) 181–243 mm SVL in males, 183 mm in the single female; (14) 30–58 mm CL in males, 35 in the single female.

Comparisons. *Ninia guytudori* sp. nov. is compared to other species of the genus previously subsumed under *N. atrata sensu lato* (differences summarized in Table 1). The new species differs from all of them by having a white nuchal collar merged with the white lip coloration (Fig. 4a), immaculate throat and chin shields (Fig. 5b, d), and ventral surface of body immaculate white (Fig. 3b). In *N. schmidti* comb. nov., the throat and chin shields are obscured by dark brown pigment

Figure 3. Female holotype of *Ninia guytudori* sp. nov. JMG 1327 in (**a**) dorsal and (**b**) ventral view.

Table 1. Differences in coloration, scale counts, and size between snakes of the genus *Ninia* inhabiting western Ecuador. The range of each continuous variable is from our own sample, Angarita-Sierra and Lynch (2017), and Angarita-Sierra (2018). The numbers in parentheses represent the sample size.

Variable	*Ninia guytudori* sp. nov.		*Ninia schmidti* comb. nov.		*Ninia teresitae*	
Throat and chin shields	Immaculate		Obscured by dark brown pigment		Irregularly spotted	
Supralabials	White (juveniles) or white with faint dark speckling		Partly or entirely black or dark gray		Partly or entirely black or dark gray	
Nuchal collar in adults	Present, entirely white or suffused with dark speckling		Absent; if present, obscured by dark smudges and spots		Absent	
Nuchal collar in juveniles	White, immaculate, connected with white supralabials, creating a "bridle"		Present, with black spots		White with black spots, no "bridle"	
Supralabials	White (juveniles) or white with faint dark speckling		Partly or entirely black or dark gray; dingy white in ZMH R10390		Partly or entirely black or dark gray	
Ventral surfaces of body	Immaculate white		Immaculate to heavily obscured by dark pigment		Irregularly spotted, speckled, or heavily obscured by dark pigment	
Pocket-shaped structure at the base of the hemipenial body	Absent		Absent		Present	
Sex	Males (n = 4)	Females (n = 1)	Males (n = 4)	Females (n = 4)	Males (n = 10)	Females (n = 15)
Maximum SVL	243 mm	183 mm	283 mm	409 mm	382 mm	346 mm
Ventral scales	130–138	138	138–144	139–155	143–156	148–160
Subcaudal scales	48–51	44	50–57	46–53	57–69	53–63

Table 2. Partition scheme and models of evolution used in phylogenetic analyses. Numbers in parentheses indicate codon position.

Partition	Best model	Gene regions	Number of aligned sites
1	GTR+I	16S, CYTB(3), ND4(1), RAG1(1)	1517
2	HKY+I	CYTB(1), ND4(2), RAG1(2)	740
3	HKY+G	CYTB(2), ND4(3)	453
3	K80	RAG1(3)	285

(Fig. 5f, h), the supralabials are partly or entirely black or dark gray (Fig. 4b), nuchal collar absent in adults (Fig. 5e, g), and ventral surfaces usually heavily obscured by dark pigment. *Ninia guytudori* sp. nov. further differs from *N. teresitae* by having a lower number of ventrals in males (130–138 vs 143–156), presence of a "white bridle," and belly not irregularly spotted, speckled, or heavily obscured by dark pigment. The cis-Andean *N. hudsoni* has black lips and dorsal scales arranged in 21 or 23 rows at mid-body (Suppl. material 1; Camper et al. 2021). *Ninia guytudori* sp. nov. differs from trans-Andean populations of *N. atrata* by having a white (instead of red, orange, or yellow) nuchal collar (Angarita-Sierra 2009), ventral surface of tail obscured by dark gray pigment (instead of uniformly cream; Angarita-Sierra and Lynch 2017), nasal divided, and by having the sulcate surface of the hemipenial body ornamented with a large basal hooked spine (photo of QCAZR 11960 depicted in Guerra-Correa 2020).

Figure 4. Lateral views of the head in two species of *Ninia*: (**a**) *N. guytudori* sp. nov. JMG 1327; (**b**) *N. schmidti* comb. nov. TH 503.

Description of holotype. Subadult female, 218 mm TL; 35 mm CL; 183 mm SVL; CL/SVL ratio 0.19; head distinct from body; HL 11.4 mm; HW 6.6 mm; rostral wider than high; internasals wider than long (1.2 × 0.6 mm); internasal suture 0.6 mm; prefrontals longer than internasals, as wide as long (1.9 × 1.9 mm; suture 1.9 mm); frontal U-shaped and as long as wide (2.6 × 2.6 mm); parietals longer than wide (4.1 × 2.2 mm); interparietal suture 2.7 mm; supraoculars 1/1, each longer than wide (1.2 × 0.8 mm), entering orbit and contacting postocular; nasal scales 2/2 where anterior nasal scale contacts internasal, rostral, first supralabial, and posterior nasal in contact with loreal, prefrontal, internasal, first and second supralabials; loreal single, longer than high (1.7 × 1.1 mm), entering orbit and in contact with 2nd and 3rd supralabials; postoculars 2/2; temporal formulae 1+2, anterior temporal scale 1.4× longer than lower posterior temporal; anterior temporal in contact with 5th and 6th supralabials; supralabials 7/8; 3rd–4th or 4th and 5th entering orbit, 5th in contact with postocular; infralabials 7/8, 1st–4th/1st–5th in contact with two pairs of chin shields; dorsal scales in 19/19/19 rows, keeled, strongly striated, lacking apical pits; ventrals 138; divided subcaudals 44; cloacal plate undivided.

Natural history. Specimens of *Ninia guytudori* sp. nov. have been found active at night on leaf-litter in old-growth cloud forest. During the daytime, they have been found hidden under rotten logs. When threatened, individuals flatten the body and tail (Fig. 8b).

Distribution. *Ninia guytudori* sp. nov. is endemic to an estimated area of 3,432 km^2 along the Pacific slopes of the Andes in northwestern Ecuador. The species is known from 11 localities (listed in Suppl. material 3) and has been recorded at elevations 1190–1676 m above sea level (Fig. 2).

Etymology. The specific epithet *guytudori* is a patronym honoring Guy Tudor, an all-around naturalist and scientific illustrator with a deep fondness for birds and all animals, in recognition of the impact he has had on the conservation of South America's birds through his artistry. For many years, Tudor and Bob Ridgely partnered in the preparation of numerous well-regarded volumes on the Neotropical avifauna.

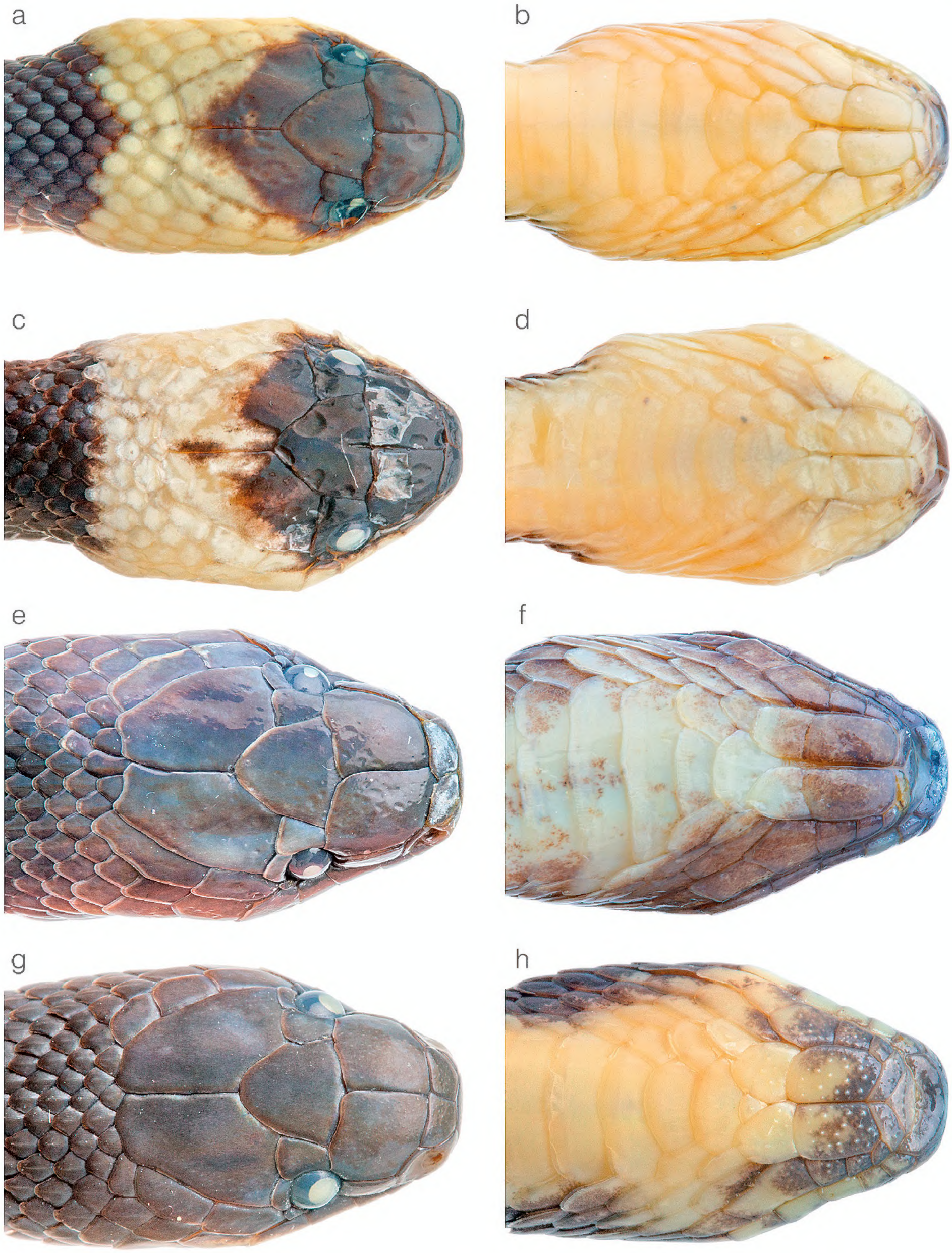

Figure 5. Dorsal and lateral views of the head in two species of *Ninia*: **(a, b)** *N. guytudori* sp. nov. JMG 1327; **(c, d)** *N. guytudori* sp. nov. SC 005; **(e, f)** *N. schmidti* comb. nov. SC 095; **(g, h)** *N. schmidti* comb. nov. SCA 1374.

Conservation status. We consider *Ninia guytudori* sp. nov. to be included in the Near Threatened conservation category following the IUCN criteria (IUCN 2012), because the species has been recorded in more than 10 localities (listed in Suppl. material 3) and it is distributed over an area which retains the majority (~53%) of its

Figure 6. Photographs of some specimens of *Ninia guytudori* sp. nov. in life: (**a**) from Río Manduriacu Reserve, Imbabura province; (**b**) from Santa Lucía Cloud Forest Reserve, Pichincha province. Photos by Jose Vieira.

Figure 7. Lateral views of some specimens of *Ninia* from western Ecuador in life: (**a**) *N. schmidti* comb. nov. SCA 1446 from Buenaventura Reserve, El Oro province; (**b**) *Ninia guytudori* sp. nov. from Santa Lucía Cloud Forest Reserve, Pichincha province. Photos by Jose Vieira.

Figure 8. Dorsal views of some specimens of *Ninia* from western Ecuador in life: **(a)** *N. schmidti* comb. nov. SCA 1446 from Buenaventura Reserve, El Oro province; **(b)** *Ninia guytudori* sp. nov. from Santa Lucía Cloud Forest Reserve, Pichincha province. Photos by Jose Vieira.

forest cover (MAE 2012). Therefore, the species is facing no major immediate extinction threats. However, some populations are likely to be declining due to deforestation by logging and large-scale mining, especially in the province Imbabura (Guayasamin et al. 2019), where only two populations of the species are known.

Ninia schmidti (Jan, 1862), comb. nov.

Figs 4b, 5e–h, 7a, 8a, 9, 10, 11, 12

Streptophorus sebae schmidti Jan, 1862: 27. Holotype ZMH (destroyed), from Guayaquil.

Ninia spilogaster Peters, 1881: 49. Holotype ZMB (not located), from Ecuador.

Neotype. ZMH R10390 (Fig. 9), adult female collected by Bi. Jansen between 1901 and 1902 at Guayaquil, Guayas province, Ecuador.

Proposed standard English name. Schmidt's Coffee-Snake.

Proposed standard Spanish name. Culebra cafetera de Schmidt.

Diagnosis. *Ninia schmidti* comb. nov. is placed in the genus *Ninia*, as diagnosed by Dunn (1935), based on phylogenetic evidence (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 19/19/19 keeled dorsals; (2) two postoculars; (3) loreal 1.4–2.3 × longer than high; (4) temporals 1+2; (5) seven supralabials, third and fourth contacting orbit; (6) seven or eight infralabials, first four or five contacting chin shields;

Figure 9. Adult female neotype of *Ninia schmidti* comb. nov. ZMH R10390 in (**a**) dorsal and (**b**) ventral view. Photos by Jakob Hallermann.

(7) usually two rows of chin shields; (8) one or two preventrals; (9) 138–144 ventrals in males, 139–155 in females; (10) 50–57 subcaudals in males, 46–53 in females; (11) dorsal ground color uniformly black without a white nuchal collar (Fig. 10); (12) ventral surfaces of adults obscured with dark pigment particularly along the posterior edge of each ventral scale (Figs 9b, 11a), immaculate white in some juveniles (Fig. 11b); (13) 167–283 mm SVL in males, 230–409 mm in females; (14) 42–61 mm CL in males, 53–84 mm in females.

Comparisons. *Ninia schmidti* comb. nov. is compared to other species of the genus previously subsumed under *N. atrata sensu lato* (differences summarized in Table 1). The new species differs from most of them (particularly from *N. guytudori* sp. nov.) by having ventral surfaces obscured by dark brown pigment particularly along the posterior edge of each ventral scale (Figs 9b, 11a), throat and chin shields obscured by dark brown pigment (Fig. 5f, h), supralabials partly or entirely black or dark gray (dingy white in ZMH R10390), and white nuchal collar in adults absent (Figs 4a, 5e, g) or faint and obscured by dark pigment (ZMH R10390). This species resembles *N. teresitae*, from which it differs by having a lower number of subcaudals and by lacking a pocket-like structure at the base of the hemipenial body (Table 1). The cis-Andean *N. hudsoni* has an immaculate white belly and dorsal scales arranged in 21 or 23 rows at mid-body (Suppl. material 1; Camper et al. 2021). *Ninia schmidti* comb. nov. differs from trans-Andean populations of *N. atrata* by lacking a nuchal collar in adults, ventral surface of tail obscured by dark gray pigment (instead of uniformly cream; Angarita-Sierra and Lynch 2017), nasal divided, and by having the sulcate surface of the hemipenial body ornamented with a large basal hooked spine (Fig. 12).

Hemipenial morphology. (n = 2; Fig. 12) Everted and inflated, the organ is cylindrical, weakly bilobed, semicalyculate and capitate. Sulcus spermaticus centrifugal, bifurcate and with walls strongly defined, bifurcation occurs proximal to the midpoint of the hemipenial body; sulcus spermaticus branch runs to lobe tips; capitation crotch located just above the bifurcation point of sulcus spermaticus (sulcate side). Region between capitation groove and lobe tips as well as entire intrasulcar region of the globular lobes homogenously ornamented with small calyces and densely packed spines. In sulcate view, base of hemipenial body surface with minute mesial spinules, but otherwise lacking medium-sized hooks. In lateral and asulcate views, base of hemipenial body covered with 2–5 rows of medium-sized hooked-shaped spines arranged in an inverted "V" pattern and one large

Figure 10. Dorsolateral views of some specimens of *Ninia schmidti* comb. nov. in life: (**a**) SCA 1446 from Buenaventura Reserve, El Oro province; (**b**) TH-503 from Vía a Tembelé, Bolívar province.

basal hook larger than any other spine on the hemipenial body; hooks replaced below by minute spicules towards the base of the organ.

Description of neotype. Adult female, 283 mm TL; 53 mm CL; 230 mm SVL; CL/SVL ratio 0.23; head distinct from body; HL 12.5 mm; HW 7.4 mm; rostral wider than high; internasals wider than long (1.4 × 0.9 mm); internasal suture 0.9 mm; prefrontals longer than internasals, as wide as long (2.3 × 2.3 mm; suture 2.1 mm); frontal shield-shaped and wider than long (2.8 × 2.6 mm); parietals longer than wide (4.0 × 2.5 mm); interparietal suture 2.4 mm; supraoculars 1/1, each longer than wide (1.3 × 0.9 mm), entering orbit and contacting postocular; nasal scales 2/2 where anterior nasal scale contacts internasal, rostral, first supralabial, and posterior nasal in contact with loreal, prefrontal, internasal, first and second supralabials; loreal single, longer than high (1.7 × 1.2 mm), entering orbit and in contact with 2nd and 3rd supralabials; postoculars 2/2; temporal formulae 1+1, anterior temporal scale 2× longer than posterior temporal; anterior temporal in contact with 4th to 7th supralabials; supralabials 7, 3rd–4th entering orbit, 5th in contact with postocular; infralabials 8, 1st–5th in contact with one pair of chin shields; dorsal scales in 19 rows, keeled, strongly striated, lacking apical pits; ventrals 144; divided subcaudals 46; cloacal plate undivided.

Natural history. Specimens of *Ninia schmidti* comb. nov. have been found active at night on leaf-litter in old-growth to heavily disturbed evergreen lowland forest and seasonally dry forests. Regdy Vera (pers. comm. to AA)

Figure 11. Ventral views of some specimens of *Ninia schmidti* comb. nov. in life: (**a**) SCA 1374B from Buenaventura Reserve, El Oro province; (**b**) SCA-1374 from Buenaventura Reserve, El Oro province.

reports that in Manabí province, this species is common under leaf-litter in humid soil and under fallen trunks, particularly in cacao plantations. In captivity, SC 095 consumed earthworms and leeches, but rejected slugs and land flatworms of the family Geoplanidae. This specimen laid a clutch of two eggs. Field observations by Vera suggest that this species does not tolerate dry, open areas.

Distribution. *Ninia schmidti* comb. nov. is endemic to an estimated area of 42,281 km^2 along the Chocoan–Tumbesian transition area in western Ecuador. The species is known from 8 localities (listed in Suppl. material 3) and has been recorded at elevations 46–1843 m above sea level (Fig. 2).

Etymology. The specific epithet *schmidti* is a patronym honoring Philipp Moses Paul Frederich Schmidt (1800–1869/1873), a physician in Hamburg best known for his work on sea snakes (Beolens et al. 2011).

Conservation status. We consider *Ninia schmidti* comb. nov. to be included in the Near Threatened conservation category following the IUCN criteria (IUCN 2012) because the species' extent of occurrence is estimated to be greater than the 20,000 km^2 threshold needed to qualify for the Vulnerable category. Unfortunately, out of the eight localities where *N. schmidti* comb. nov. is known to occur, two represent historical populations and five are of singleton individuals. The species occurs as fragmented populations and occurs over an area where most (~80%) of the forest cover has been transformed into plantations and human settlements (MAE 2012). Therefore, *N. schmidti* comb. nov. may qualify for a

Figure 12. Hemipenial architecture of *Ninia schmidti* comb. nov. in sulcate, lateral, and asulcate views: (**a**) SCA 1374 from Buenaventura Reserve, El Oro province; (**b**) SCA 1446 from Buenaventura Reserve, El Oro province.

threatened category soon if its habitat continues to be destroyed. Fortunately, the species occurs in one protected area: Buenaventura Reserve.

Discussion

This work marks the second attempt elucidating the identities of Ecuadorian snakes labeled as *Ninia atrata*. It provides evidence of the existence of three species of *Ninia* in western Ecuador, none of which can be allocated to *N. atrata sensu stricto* based on molecular evidence and color pattern characteristics. Thus, we remove the latter species from the list of Ecuadorian herpetofauna. We propose to use the name *Ninia schmidti* comb. nov. over *N. spilogaster*, simply based on priority. We agree with Dunn (1935), that both names correspond to the same taxon based on the description of *N. spilogaster* by Peters (1881), in which he mentions a darkened ventral coloration and lack of a white nuchal collar. We suggest that the status of *Streptophorus drozii* and *S. lansbergi* as synonyms of *N. atrata sensu stricto* be maintained pending a more detailed study of Venezuelan populations.

The distribution of the three species of *Ninia* in western Ecuador mirrors the distribution of other trans-Andean squamates in the country. For example, *N. teresitae* is a strictly Chocoan snake species and its distribution resembles that of other snakes endemic to this biome

(MECN 2010). The distribution of *N. guytudori* sp. nov. corresponds with lower-montane forests in the area between the Río Mira and Toachi valleys, the area in Ecuador where these two vegetation zones are wider and closer to the Equatorial line. *Ninia guytudori* sp. nov. can be included in the list of species that share this pattern (Arteaga et al. 2016). *Ninia schmidti* comb. nov. shares it distribution with other reptiles that are restricted to the transition area between the humid Chocó rainforests and the Tumbesian dry forests, including *Enyalioides touzeti* Torres-Carvajal et al. 2008, *Anadia buenaventura* Betancourt et al., 2018, *Dipsas bobridgelyi* Arteaga et al., 2018, *Sibon bevridgelyi* Arteaga et al., 2018, and *Anolis nemonteae* Ayala-Varela et al., 2021. We believe this shared pattern suggest the existence of a unique biogeographical province between the humid Chocó rainforests and the Tumbesian dry forests.

We suggest that based on the number of locality records included in the analyses (Suppl. material 3), the environmental niche models and corresponding distribution areas (Fig. 2) for the three species of *Ninia* occurring in western Ecuador are in themselves diagnostic and ecologically informative. We found no sympatry between the sister species *Ninia schmidti* comb. nov. and *Ninia guytudori* sp. nov., suggesting a pattern of allopatric speciation.

Although this work advances our knowledge on Ecuadorian *Ninia*, it is still far from complete. First, the paraphyly of *N. atrata* still needs to be resolved, including the status of trans-Andean populations assigned to this species. Second, the hemipenial morphology of *N. guytudori* sp. nov. has not been described in detail. Third the relationship between Ecuadorian *N. hudsoni* and those from the type locality (Guyana) is still uncertain, but worthy of study, particularly since populations of this species throughout the Amazon are known to be discontinuous.

We suggest that any future work focused on the systematics of the *Ninia atrata* species complex include a comprehensive sampling of molecular characters. Such work would gain much clarity by sampling species of *Ninia* occurring on the Guianas and along the Río Magdalena valley in Colombia. Until then, we hope that our work helps guide future studies into the biogeography of this charismatic group of colubrids.

Author contributions

Conceived and designed the work: AA. Performed the analyses: AA. Gathered morphological data: AA, KJH. Analyzed the data: AA. Wrote the paper: AA, KJH.

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Appendix 1

Table A1. List of PCR and sequencing primers and their respective PCR conditions (denaturation, annealing, extension and number of corresponding cycles) used in this study. All PCR protocols included an initial 3-min step at 94 °C and a final extension of 10 min at 72 °C.

Locus	Primer	Sequence (5'-3')	Reference	PCR profile:
16S	16Sar-L	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)	94 °C (45 sec), 53 °C (45 sec), 72 °C (1 min) [×30]
	16Sbr-H-R	CCGGTCTGAACTCAGATCACGT		
Cytb	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	Burbrink et al. (2000)	94 °C (1 min), 58 °C (1 min), 72 °C (2 min) [×30–36]
	H16064	CTTTGGTTTACAAGAACAATGCTTTA		
ND4	ND4	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al. (1994)	94 °C (25 sec), 56 or 60 °C (1 min), 72 °C (2 min) [×25–30]
	Leu	CATTACTTTTACTTGGATTTGCACCA		
RAG-1	MartFL1	AGCTGCAGYCARTAYCAYAARATGTA	Barlow et al. 2009	95 °C (30 sec), 55 °C (45 sec), 72 °C (1 min) [×35]
	AmpR1	AACTCAGCTGCATTKCCAATRTCA		

Supplementary material 1

Morphological and locality data

Authors: Alejandro Arteaga, Kyle J. Harris
Data type: xlsx
Explanation note: Morphological and locality data for specimens of *Ninia* examined, either directly or indirectly through digital photographs. Codes: SVL = snout-vent length, CL = Caudal length, M = Male, F = Female.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/evolsyst.7.112476.suppl1

Supplementary material 2

GenBank accession numbers

Authors: Alejandro Arteaga, Kyle J. Harris
Data type: xls
Explanation note: GenBank accession numbers for loci and terminals of taxa and outgroups sampled in this study. Novel sequence data produced in this study are marked with an asterisk (*).
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/evolsyst.7.112476.suppl2

Supplementary material 3

Locality data used to create distribution maps

Authors: Alejandro Arteaga, Kyle J. Harris
Data type: xlsx
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
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